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A Mixture of Thyme, Quillaja, and Anise at Different Nutrient Density on Growth Performance, Nutrient Digestibility, Meat Quality, Organ Weight, Cecal Bacteria, Excreta Moisture, and Bone Contents in Broiler Chicks

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ABSTRACT The experiment was carried out on 648 Ross 308 broilers (40.4±0.2 g; 1 d) chickens. It lasted for 28 days to evaluate the effects of phytogenic feed additive (PFA) on growth performance, nutrient digestibility, cecum microbiota, bone minerals contents, meat quality and internal organ weights. The chickens were haphazardly divided into any 1 of 6 dietary treatments (6 replicate pens/treatment; 18 broilers/pen). The experimental diets were: CON, control; T1, CON + 150 ppm PFA; T2, CP 0.5% reduced from basal diet; T3, T2 + 150 ppm PFA; T4, Ca 0.07% reduced + available P (aP) 0.065% reduced from basal diet; T5, T4 + 150 ppm PFA. Overall, broilers fed with T3 diet tended to have higher (P=0.08) body weight gain (BWG), and lower (P=0.05) feed conversion ratio (FCR) than T2 diet. The apparent total tract digestibility (ATTD) of P was lower (P=0.03) in T2 as compared to CON and it was higher (P=0.02) in T3 as compared to T2. There were no differences in meat quality, relative organ weight, and cecum microbiota (P>0.05). The supplementation of PFA showed trends in improvement in ash content in the bone of birds fed T1, T2 and T4 diets compared with CON. Likewise, the supplementation of PFA tended to increase (P=0.07) Ca content in the bone of birds fed T1 diet compared to CON. In conclusion, dietary supplementation with 150 ppm PFA could improve BWG in birds fed protein reduced diet, improve apparent total tract digestibility (ATTD) of P, and the bone Ca contents in broilers.

(Key words: broilers, growth performance, meat quality, mineral reduced diet, protein reduced diet, phytogenic feed additive)

INTRODUCTION

In poultry production, the reduction of dietary protein, minerals, and energy while maintaining poultry performance and health has been considered as one of the strategies to reduce feed cost. However, the reduction of protein, minerals or energy may have an adverse effect on performance and health. To compensate the undesirable effect of reduced protein and minerals the addition of certain emulsifier, nucleotides, organic acids, phytogenic, and nutraceutical are found to possess favorable results. For instance, Amad et al. (2011) reported that addition of 150, 750 or 1,500 mg/kg phytogenic feed additive (PFA) linearly increased ileal digestibility of crude fat, crude protein, calcium and phosphorous. Phytogenic is a heterogeneous and plant derived feed additives that contain herbs, spices, fruit, and other plant parts (Windisch et al., 2008). These phytogenic are enriched with biologically active ingredients such as phenolic compounds, tannins, bitter compounds, organic acids, vitamins, minerals, enzyme and mucilage (Dzida et al., 2015). Different kinds of phytogenic have been used for human and veterinary medicines for many decades and in recent years phytogenic as a feed additive has attracted deep attention in feed industry too (Alagawany et al., 2016). The PFA fed to animals have been found to improve growth performance, feed conversion ratio, as well as meat quality of carcass (Dhama et al., 2015). Besides improving growth, phytogenic as feed additive has anti-oxidant property (Alagawany et al., 2016). It was found helpful in enhancing the gastrointestinal morphology of weanling piglets (Upadhaya et al., 2016). Platel and Srinivasan (2004) found the essential oil with thymol as a lead component has been detected to motivate digestive secretions like salivary amylase in humans. Whereas in rats, it is found to enhance gastric juice, bile acids, pancreatic enzymes, and intestinal mucosa. There was a significant increase of body weight gain in broiler chickens when it was fed with 0.5 g/kg anise seed

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that contained anothole as a vital constituent. However, no significant difference was observed in feed intake and feed conversion ratio when the broilers were fed with 5,000 ppm Labiatae extract (LE) from sage, thyme, and rosemary and 200 ppm of essential oil extract (EOE) from oregano, cinnamon, and pepper at 14 to 21 d of age (Hernandez et al., 2004). We hypothesized that if protein and mineral reduced diet is supplemented with PFA, it may help to overcome the undesirable effect of reduced nutrient thereby reducing feed cost and maintaining animal health and performance.

Thus, our study was focused to evaluate the efficacy of phytogenic blend product on growth performance, nutrient digestibility, caecum microbiota, meat quality as well as bone mineral content in broilers.

MATERIALS AND METHODS

1. Experimental Design, Birds, Housing and Diets

The procedures applied for the experiment was as per the guidelines given by the Animal Care and Use Committee of Dankook University. Six hundred and forty-eight Ross 308 mixed-sex 1d-old broiler chicks having an average BW of 40.4±0.2 g were used in the experiment which lasted for 28 days. Chicks were haphazardly divided into 1 of the 6 experimental diets (6 replicate pens/ treatment; 18 chickens/pen). Chickens were grown in a room of 33±1℃ for the first 3 days and the temperature was reduced gradually until 24℃ maintaining humidity around 60% for the rest of the experiment. Stainless steel pens (1.75×1.55 m) of identical size were managed for chickens housing. Diets fed to chickens were: CON (basal diet), T1 (basal diet+150 ppm phytogenic blend), T2 (basal diet with CP 0.5% reduced), T3 (T2+150 ppm phytogenic blend), T4 (basal diet with Ca 0.07% and P 0.065% reduced) and T5 (T4+150 ppm phytogenic blend). The basal diet was prepared according to National Research Council recommendations (NRC, 1994) (Table 1), whereas the treatment diets were prepared by reducing CP, Ca and P. Easy access to feed and water was managed during the trial. The phytogenic blend employed in this experiment consisted of essential oils of quillaja (30%), anise (20%), thyme (17%) and wheat flour as a carrier (33%).

2. Sampling and Measurements

Body weight (BW) and feed intake (FI) were measured on 1st, 7th, 21st, and 28th day to measure the body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N_2), energy (E), ash, calcium (Ca), and phosphorus (P) were determined by adding 0.2% chromium oxide as an inert gas indicator in the diet for 1 week before fecal sample collection. On d28 excret a samples were grabbed from each pen and stored at −20℃. For chemical analysis, the feed and fecal samples were dried in an electrical oven for 72 hr at a temperature of 60℃. After the drying process was completed, the samples were ground and filtered through a 1-mm sieve. Then, the analysis was carried out as per guidelines of AOAC International (2005). Nitrogen was analyzed by Kjeltec 2300 Nitrogen Analyzer (Foss Tecator AB, Höganäs, Sweden). Gross energy (GE) was calculated by computing the heat generated by combustion, using a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL). Chromium was detected by UV absorption spectrophotometry (Shimadzu, UV-1201, Kyoto, Japan) by the guidelines of Williams et al. (1962).

At the final day of the trial, chickens were weighed and slaughtered by cervical dislocation. Three chickens per replicate were randomly chosen to collect caecal contents for the enumeration of *Lactobacillus* and *Clostridium*. The caecal digesta was collected (2 to 3 cm ahead of cloaca) and pooled on pen basis for microbiota count. One gram of composite caecal digesta content from each pen per treatment was moderated with 9 mL broth containing 1% peptone (Becton, Dickinson and Co., Franklin Lakes, NJ) and then stirred for homogenization. The *Lactobacilli* medium III agar plates were used for isolation of *Lactobacillus* and clostridial medium agar plates were used for isolation of *Clostridium perfringens*. The caecal samples were undergone through 10-fold dilutions (in 1% peptone solution). It was placed on *Lactobacilli* medium III agar plates and incubated for 48 hr at 39℃ in an anaerobic environment. Similarly, the samples were placed on clostridial medium agar plates and placed in an incubator for 24 hr at 37℃. The viable counts of *Lactobacillus* and *Clostridium perfringens* were noted immediately after removal from the incubator.

Table 1. Composition and analyzed nutrients of experimental diet as fed basis

Ingredients (g/kg)		Starter			Grower			Finisher		
		Basal diet	CP reduced diet	Ca+P reduced diet	Basal diet	CP reduced diet	Ca+P reduced diet	Basal diet	CP reduced diet	Ca+P reduced diet
Corn		454.8	477.0	463.6	366.9	417.0	376.7	408.0	416.0	412.4
Wheat		100.0	100.0	100.0	200.0	170.0	200.0	200.0	200.0	200.0
Soybean meal (CP 48%)		342.5	324.3	342.3	336.2	317.0	334.8	254.8	239.9	254.8
Rapeseed meal		0	0	0	0	0	0	35.0	35.0	35.0
Corn gluten meal		20.0	20.0	19.8	0	0	0	0	0	0
Tallow		19.0	14.3	16.2	55.4	50.9	52.4	60.1	57.2	60.1
Soybean oil		15.0	15.0	15.0	0	0	0	0	0	0
Limestone		10.6	10.6	11.4	11.2	11.2	12	11.7	12.0	13.0
Dicalcium phosphate		22.3	22.3	15.9	19.0	19.0	12.7	18.4	18.4	12.0
Salt		3.5	3.5	3.5	3.2	3.2	3.2	2.9	3.0	2.9
DL-Methionine (88%)		4.6	4.6	4.6	3.9	4.1	3.9	4.1	4.2	4.1
L-Lysine-HCl (78.4%)		4.2	4.9	4.2	1.5	2.0	1.6	2.0	2.5	2
Threonine (98.5%)		1.7	1.7	1.7	0.9	1.1	0.9	1.2	10.0	1.2
Vitamin premix[1]		0.7	0.7	0.7	0.3	3.0	0.3	0.3	0.3	1.0
$CuSO_4$		0	0	0	0.4	0.4	0.4	0.4	0.4	0.4
Trace mineral premix[2]		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Xylanase[3]		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calculated nutritional content	ME (kcal/kg)	3,015	3,015	3,013	3,114	3,110	3,112	3,180	3,180	3,180
	CP (g/kg)	221	216	221	205	200	205	185	180	185
	Lysine (g/kg)	14.5	14.5	14.5	12.2	12.2	12.2	11	11	11
	Met + Cys (g/kg)	10.6	10.6	10.6	9.5	9.5	9.5	9.3	9.2	9.3
	Ca (g/kg)	10.5	10.5	9.79	10	10	9.3	10	10	9.3
	Available P (aP) (g/kg)	5.25	5.25	4.6	5.01	5.01	4.36	5.03	5	4.4
	Crude fat (g/kg)	55.5	55.5	52.9	72.7	69.4	70	79.6	77	80
	Crude fiber (g/kg)	32.4	32.4	32.6	32.9	32.6	33	32.5	32	33
Analyzed nutritional content	GE (kcal/kg)	3,713	3,726	3,705	3,823	3,824	3,818	3,862	3,861	3,863
	CP (g/kg)	222	216	221	206	200	206	187	181	222
	Ca (g/kg)	9.24	9.33	8.68	9.12	9.30	8.59	9.10	9.10	9.20
	P (g/kg)	6.66	6.68	6.00	6.32	6.31	5.64	6.44	6.50	6.70

[1] Provided per kg of complete diet: 5.16 mg of retinyl acetate, 94 µg of cholecalciferol, 37.5 mg of Dl-α tocopherol acetate, 2.25 mg of menadione, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of pyridoxine, 24 µg of cyanocobalamin, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of Ca-pantothenate.

[2] Provided per kg of complete diet: 37.5 mg of Fe (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H_2O$), 37.5 mg of zinc (as $ZnSO_4$), 37.5 mg of Mn (as MnO_2), 0.83 mg of I (as KI), 0.23 mg. of Se (as $Na_2SeO_3 \cdot 5H_2O$).

[3] Xylanase, 7,000 IU (Nutrase xyla).

For bone ash percentage determination, tibia bone samples from 3 birds per replication were randomly chosen and put in an electrical oven (105℃, 16 hr). Fat contained in the bone was eliminated by petroleum-ether extraction. The bones were dried again at 105℃ and weighed. When it was put overnight in a muffle furnace (600℃), it converted to ash. Ash weight was noted and dissolved in HCl (Miles et al., 2001). Ca and P were detected by using a spectrometer (Optima 2000 DV, Perkin-Elmer, Uberlingen, Germany). The values thus obtained were listed on a DM basis.

Four chickens per replication per treatment were randomly chosen for meat quality analysis. The breast meat, abdominal fat, gizzard, spleen, liver, and the bursa of fabricius were carefully removed by trained personnel. The organs weight were expressed as a percentage of BW. Hunter L* (lightness), a* (redness), and b* (yellowness) of breast meat were analyzed by using a Minolta CR410 Chroma Meter (Konica Minolta Sensing Inc., Osaka, Japan). The drip loss % of the meat was evaluated on 1st, 3rd, 5th, and 7th day afters slaugh- tering by Honikel (1998) method.

3. Statistical Analysis

All the experimental data were statistically evaluated by SAS (GLM Procedure) as a complete randomized block design (SAS Inst. Inc., Cary, NC 1996). Pre-planned contrasts were applied to examine the individual effect of PFA supplementation to basal diet as well as protein and mineral reduced diets. Variability of the results was listed as the least squares means SE and statistical significance was considered at $P<0.05$.

RESULTS

1. Growth Performance

During the experimental periods, all of the birds were healthy. The growth performance parameters (BWG, FI and FCR) were unaffected ($P>0.05$) when PFA was supplemented to basal, protein reduced and mineral reduced diets during starter, grower and finisher phase. However, during the overall period, the supplementation of PFA tended to show higher ($P=0.08$) BWG and lower ($P=0.05$) FCR in T3 than T2 as shown in Table 2.

Table 2. The effect of PFA on growth performance in broilers

Items (days)		CON[1]	T1	T2	T3	T4	T5	SE[2]	*P*-value				
									CON vs T1	CON vs T2	CON vs T4	T2 vs T3	T4 vs T5
1~7	BWG (g)	158.0	159.8	159.2	161.3	162.8	164.2	3.120	0.69	0.81	0.47	0.64	0.77
	FI (g)	184.5	186.3	186.1	188.5	189.7	196.0	3.780	0.73	0.76	0.46	0.67	0.25
	FCR	1.168	1.166	1.169	1.169	1.165	1.194	0.036	0.86	0.94	0.88	0.94	0.53
8~21	BWG (g)	446.0	459.3	416.8	447.5	424.6	420.0	15.800	0.56	0.20	0.95	0.18	0.84
	FI (g)	682.5	677.6	684.3	683	698.5	678.2	9.800	0.73	0.90	0.96	0.93	0.16
	FCR	1.530	1.475	1.642	1.527	1.645	1.615	0.060	0.46	0.17	0.97	0.16	0.85
22~28	BWG (g)	800.0	803.8	795.2	801.8	798.2	806.5	11.100	0.81	0.76	0.91	0.67	0.60
	FI (g)	1,190	1,187	1,189	1,171	1,182	1,177	16.000	0.91	0.98	0.42	0.43	0.84
	FCR	1.488	1.477	1.495	1.460	1.481	1.459	0.030	0.84	0.83	0.53	0.41	0.60
Overall	BWG (g)	1,404	1,423	1,371	1,411	1,386	1,391	15.000	0.38	0.14	0.75	0.08	0.82
	FI (g)	2,056	2,051	2,060	2,042	2,070	2,052	17.000	0.83	0.89	0.57	0.48	0.45
	FCR	1.464	1.441	1.503	1.447	1.494	1.475	0.020	0.35	0.18	0.50	0.05	0.53

[1] CON, control; T1, CON + 150 ppm PFA; T2, CON - CP 0.5% reduced; T3, T2 + 150 ppm PFA; T4, CON - Ca 0.07% reduced and aP 0.065% reduced; T5, T4 + 150 ppm PFA.

[2] Pooled standard error.

2. Nutrient Digestibility and Caecal Microbiota

The apparent total tract digestibility (ATTD) of DM, N, energy, ash and Ca were unaffected (*P*>0.05) when PFA was supplemented to basal, protein reduced and mineral reduced diets (Table 3). However, the ATTD of P was lower (*P*= 0.03) in T2 compared to CON and it was higher (*P*=0.02) in T3 as compared with T2. The *Lactobacillus* and *Clostridium* count obtained from caecal digesta were also unaffected (*P*>0.05) when PFA was supplemented to basal, protein reduced and mineral reduced diets (Table 3).

3. Mineral Contents in Bone

As shown in Table 4, the supplementation of PFA showed trends of improvement in ash content in the bone of birds fed T1, T2 and T4 diets compared with CON. Similarly, the supplementation of PFA tended to increase (*P*=0.07) Ca content in the bone of birds fed T1 diet compared to CON. However, P content in bone was unaffected (*P*>0.05) with PFA supplementation to basal, protein reduced and mineral reduced diets.

4. Meat Quality and Relative Organ Weight

The meat quality and relative organ weight were unaffected (*P*>0.05) when PFA was supplemented to basal, protein reduced and mineral reduced diets (Table 5).

DISCUSSION

Table 3. The effect of PFA on nutrient digestibility and excreta microbiota in broilers[1]

Items (%)		CON[1]	T1	T2	T3	T4	T5	SE[2]	*P*-value				
									CON vs T1	CON vs T2	CON vs T4	T2 vs T3	T4 vs T5
DM		76.13	76.50	76.84	76.28	76.70	76.80	0.95	0.81	0.60	0.91	0.68	0.94
N		62.35	62.52	62.80	62.31	62.59	62.50	1.87	0.95	0.86	0.99	0.85	0.97
Energy		78.67	78.35	78.02	77.56	77.87	78.64	0.94	0.81	0.63	0.42	0.74	0.57
Ash		45.69	46.46	46.49	46.66	45.56	45.36	4.90	0.91	0.90	0.89	0.98	0.98
Ca		51.51	56.60	52.57	51.63	54.21	56.85	2.13	0.10	0.73	0.97	0.76	0.39
P		55.09	55.23	47.02	55.15	49.02	54.20	2.47	0.97	0.03	0.98	0.02	0.15
Caecal microbiota (log_{10}cfu/g)	*Clostridium perfringens*	2.39	2.30	2.41	2.31	2.32	2.28	0.09	0.49	0.92	0.54	0.48	0.77
	Lactobacillus	7.54	7.68	7.56	7.58	7.61	7.53	0.06	0.13	0.82	0.63	0.79	0.45

[1] CON, control; T1, CON + 150 ppm PFA; T2, CON - CP 0.5% reduced; T3, T2 + 150 ppm PFA; T4, CON - Ca 0.07% reduced and aP 0.065% reduced; T5, T4 + 150 ppm PFA.
[2] Pooled standard error.

Table 4. The effects of PFA on bone ash, calcium and phosphorus content in broilers[1]

Items (%)	CON[1]	T1	T2	T3	T4	T5	SE[2]	*P*-value				
								CON vs T1	CON vs T2	CON vs T4	T2 vs T3	T4 vs T5
Ash	33.00	34.30	34.49	34.47	34.30	34.70	0.53	0.09	0.06	0.06	0.98	0.6
Ca	10.06	10.76	10.13	10.38	9.71	9.89	0.27	0.07	0.83	0.4	0.53	0.64
P	5.25	5.30	5.25	5.24	5.13	5.29	0.05	0.98	0.96	0.1	0.11	0.82

[1] CON, control; T1, CON + 150 ppm PFA; T2, CON - CP 0.5% reduced; T3, T2 + 150 ppm PFA; T4, CON - Ca 0.07% reduced and aP 0.065% reduced; T5, T4 + 150 ppm PFA.
[2] Pooled standard error.

Table 5. The effect of PFA on meat quality and relative organ weight in broilers[1]

Items (%)		CON[1]	T1	T2	T3	T4	T5	SE[2]	*P*-value				
									CON vs T1	CON vs T2	CON vs T4	T2 vs T3	T4 vs T5
pH value		5.60	5.60	5.60	5.50	5.60	5.60	0.03	0.83	0.48	0.08	0.27	0.72
Breast muscle color	Lightness (L*)	55.90	55.39	55.83	55.47	55.26	55.72	1.25	0.78	0.97	0.81	0.84	0.79
	Redness (a*)	15.68	14.91	14.63	14.94	15.62	14.92	0.83	0.52	0.39	0.54	0.80	0.56
	Yellowness (b*)	14.65	15.62	15.16	16.06	15.09	16.33	0.95	0.48	0.71	0.31	0.51	0.37
WHC (%)		60.07	60.61	62.83	60.45	62.34	61.83	3.95	0.92	0.63	0.95	0.68	0.93
Drip loss (%)	1 d	2.64	2.94	2.63	2.86	2.41	2.80	0.48	0.66	0.99	0.75	0.74	0.57
	3 d	4.64	4.76	4.61	4.71	4.45	4.52	0.56	0.88	0.97	0.93	0.90	0.93
	5 d	7.81	8.69	8.41	8.39	8.45	8.59	0.67	0.37	0.53	0.55	0.98	0.87
	7 d	10.82	11.50	10.76	10.47	11.71	10.42	1.57	0.76	0.98	0.86	0.89	0.57
Breast muscle (%)		16.76	17.45	16.61	17.03	17.75	16.27	1.10	0.66	0.93	0.86	0.79	0.36
Abdominal fat (%)		1.15	1.40	1.14	1.40	1.24	1.39	0.14	0.22	0.97	0.21	0.19	0.44
Relative organ weight (%)	Liver	2.69	2.40	2.58	2.61	2.99	2.49	0.19	0.31	0.70	0.77	0.92	0.09
	Spleen	0.18	0.19	0.17	0.19	0.16	0.19	0.02	0.77	0.69	0.69	0.44	0.33
	Bursa of fabricius	0.14	0.20	0.16	0.15	0.14	0.17	0.03	0.12	0.62	0.80	0.80	0.46
	Gizzard	1.90	1.81	1.83	1.89	1.77	1.85	0.11	0.56	0.64	0.96	0.67	0.56

[1] CON, control; T1, CON + 150 ppm PFA; T2, CON - CP 0.5% reduced; T3, T2 + 150 ppm PFA; T4, CON - Ca 0.07% reduced and aP 0.065% reduced; T5, T4 + 150 ppm PFA.
[2] Pooled standard error.

The current study found no significant effect on growth performance of broilers by supplementing the diet with PFA from starter to finisher phase. On an overall, there was an improvement in BWG and FCR of birds fed with T3 as compared to T2. In an earlier study, Kamran et al. (2008) also noted that reducing CP levels worsened FCR during all growth periods except the starter periods in broilers. Paraskeuas et al. (2016) also indicated that reduced protein diet in broiler led to poorer FCR at growing period and overall period compared with basal diet. In line with our findings, some studies also reported no significant effects of PFA supplementation to basal diet on broiler performance. For instance, carvacrol, which was added at concentrations of 200 ppm to the diet, had reduced feed intake, weight gain, and feed: gain ratio compared with the control treatment (Lee et al., 2003). Similarly, no significant effect was observed with the supplementation of blends of essential oils derived from *Mentha piperita*, *Thymus vulgaris*, *Cirtus lemon* and *Carum copticum* at three different concentrations of 50, 100 and 150 mg/kg on growth performance of broiler chickens (Samadian et al., 2013). On the contrary, positive effect was noticed on the growth performance of birds fed with essential oils extracted from caraway, lemon, sage, basil, laurel, oregano, tea and thyme at the concentration of at least 100 g/ton feed showed positive effect on growth performance (Khattak et al., 2014). Also, the addition of 50 mg/kg or 100 mg/kg of garlic and onion resulted in the significant body weight gain of broiler chickens (Aji et al., 2011). There was a noticeable improvement in body weight, average daily gain, and feed conversion ratio of broiler chickens when 1 g/kg or 2 g/kg of anise seed was added to the diet. But no significant effect was noticed in feed intake (Alhajj et al., 2015). A previous study on essential oil blends (including quillaja, anise, and thyme) supplementation at the dosage of 150 ppm to duck improved the growth performance on d 21 to 42 and the overall experimental period (Gheisar et al., 2015). It has been suggested that PFA can increase the activity of some digestive enzymes such as trypsin, lipase, and amylase, which may improve FCR and BWG (Jamroz et al., 2005). However, various doses of PFA containing different amounts of active substance, bird species as well as the experimental period and the composition of the diet may be the major cause for the difference in results (Upadhaya et al., 2017).

In the current study, the digestibility of DM, N, energy, ash, and calcium for T1, T2, T3, T4 and T5 diets were similar to that of basal diet. However, the ATTD of P was significantly lower in protein reduced diet compared to CON. The ATTD of P was significantly higher in protein reduced diet supplemented with PFA compared with non-supplemented diet indicating PFA positively affected P retention. Phytogenic feed additives consisting of cinnamaldehyde, carvacrol, and capsicum oleoresin supplemented in different diets (wheat-barley-soybean meal or corn-soybean meal diets) did not enhance the apparent digestibility of crude protein and amino acids in comparison with the control diet, when fed to male Hubbard broilers (Puvaca et al., 2013). The dietary inclusion of herbs (thymol, oregano, marjoram, rosemary or yarrow) and essential oils from it increased the apparent metabolizable energy and the apparent total tract digestibility of dry matter and organic matter in broiler chicks (Cross et al., 2007). Releasing more enzymes into the gastro intestinal tract by using PFA may improve growth performance, but had no significant effects on the digestibility of nutrients in some surveys (Muhl and Liebert, 2007b). The main reason for this result may be related to the conservation of energy for the improvement in animal health and reduction in the energy required for gut maintenance such as for producing mucus (Arias and Koutsos, 2006).

In the current study, feed supplemented with PFA did not significantly alter caecal *Lactobacillus* and *Clostridium perfringens* counts in broilers compared with the PFA un-supplemented feed. On the contrary, another study found that antimicrobial characteristics of some essential oils such as carvacrol, thymol, curcumin, eugenol, and piperin have a detrimental effect on the growth of various strains of Clostridia such as *C. perfringens* (Dorman and Deans, 2000). In a previous study, an addition of 250 mg/kg essential oil of thyme and star anise as PFA resulted in a reduction of *C. perfringens* in the large intestine and decrease of *E. coli* in the small intestine of broilers (Cho et al., 2014). Cross et al. (2007) also found that the inclusion of thyme, marjoram, and rosemary in broiler diets reduced the numbers of caecal *C. perfringens*. An increase in caecal *Lactobacillus* in broiler fed with the diet containing thymol and cinnamaldehyde as essential oils were reported by Tiihonen et al. (2010). The possible reason for plant extracts such as thyme to control pathogens may be due to its hydrophobicity nature. Because of this property, these compounds can intersect the cell membrane of bacteria leading to cell membrane disintegration, finally resulting in the death of bacteria (Burt, 2004). The reason for a non-significant effect of essential oil on cecal microbiota could be the difference in the composition of essential oil blends, (inclusion of quillija powder along with thyme and anise), diet composition or the dose of PFA.

The supplementation of PFA to basal diet showed trends in an increase in Ca content in the bone. However, there were no significant effects of PFA on Ca or P contents in the bones of birds fed minerals and protein reduced diet supplemented with PFA. Onyango et al. (2003) indicated that as the levels of dietary calcium and phosphorus increased the bone mineralization concentrate also increased linearly due to an increase in the levels of calcium and phosphorus. This implies that when the levels of Ca and P in the diet decrease, the amount of calcium and phosphorus required for mineralization will also decrease.

Considering the European Food Safety Authority (EFSA), improving the quality of foodstuffs seems very necessary and using phytogenic additives may be an effective way to maintain the quality of produced meat. *Mentha spicata* (1% to 4%) in the diet was found helpful in improving the meat quality of Japanese quail (Ghazaghi et al., 2014). The dietary addition of red hot and black pepper (0.75 and 1 %) was found effective in improving the dressing percentage of broiler chickens (Al-Kassie et al., 2011). In the current study, we assumed that antioxidative characters of thyme, anise star, and quillaja could help to improve the quality of meat since it can protect the oil from oxidation. However, our present study indicated that the meat quality, breast muscle, abdominal fat, relative weights of the spleen, liver, bursa of fabricius, and gizzard were not affected in birds consuming PFA supplemented diet. Similar results were reported by Hernández et al. (2004), Demir et al. (2008) and Samadian et al. (2013). Amad et al. (2011) also summarized that internal organs such as spleen, kidney, heart, and liver were unaffected with the supplementation of PFA (thymol, carvacrol, and eugenol) to the broiler diet. It can be concluded that the main apparent effect of PFA lies in the realm of the gastro intestinal tract rather than in internal organs. In general, these responses indicate that this mixing ratio of thyme, anise, and quillaja had a slight effect on the growth performance but they cannot be regarded as a very strong potential growth enhancer in the broiler. Further investigations should place more emphasis on establishing the optimal application of such additives to evaluate the ideal dosage for optimal results.

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